

Venom and Task Specialization in *Termitopone commutata* (Hymenoptera: Formicidae)

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Abstract.—*Termitopone commutata* is a large ponerine ant species specializing in termite prey. They form raiding armies of workers that overwhelm the termite defenses, sting and inactivate termites including soldiers, and carry them back to their colony. Little natural history has been reported for this species and nothing is known of task specialization of workers. We excavated and totally censused a record-sized colony and separated individuals into categories of raiders, nest defenders, and nest workers. The amount of venom per worker in the three behavioral castes and in alate females was measured and the respective lethalities and paralyzing abilities of their venoms determined. Venom activities mirrored the needs of the three task specialists indicating matching physiology and behavior in this species.

Key words.—*Termitopone commutata*, *Neoponera*, venom, paralysis, lethality

Termitopone is a small Neotropical genus of ponerine ants that are obligate specialist predators of termites (Wheeler 1936). *Termitopone commutata* (Roger) is, as Roy Snelling would say, a "handsome beast", striking not only for the enormous 15–19 mm size of the workers, but also for its smooth and shiny black sleek appearance. Although the largest of the three species in the genus, *T. commutata* also is the most poorly known. A note on taxonomy is appropriate here. The genus has been variously identified (among others) as *Neoponera*, *Termitopone*, and *Pachycondyla*. Recently the genus was again placed in the paraphyletic "trash" genus *Pachycondyla*, apparently as a holding position until more definitive placement could be made. For clearer communication, I will use the name *Termitopone*, though the genus likely will be placed in a newly erected *Neoponera* soon (Chris Schmidt, personal communication).

Termitopone commutata appears to be an obligate predator of any *Syntermes* spp., which they capture by stinging and para-

lyzing both workers and soldiers. When a scout discovers a *Syntermes* foraging group, she lays a pheromone trail back to the nest and recruits a raiding party. A raiding column then follows the attacker to the termites where the individual ants spread out and quickly attack and paralyze the termites. Upon completion of the attack, the termites are carried back to the nest (Hermann 1968; Mill 1982, 1984). Raiding columns are reported to contain about 43 ants (20–117) and travel up to 40 m (Mill 1984). Colony populations are considered small compared to the congeneric *T. marginata* (Leal and Oliveira 1995), though no colony excavations and counts have been reported. Mill (1982) estimated one observed colony to contain 400 workers.

Workers of *T. commutata* are well-known to defend their colonies vigorously and their stings have gained some notoriety for being very painful. This algogenicity of their stings has earned them roles in human social rituals, most notably in

initiation rites of girls into womanhood among Tupi-Guarani and other peoples in Amazonia (Balee 2000). Though painful, *T. commutata* stings pale in comparison to those of *Paraponera clavata*, a species not used in female rites, but reserved among some tribes for manhood rites (Balee 2000).

The dual roles of venom in *Termitopone commutata* for both prey capture and for defense against large predators makes the species an ideal model for testing the role of venom physiology in behavior. These two venom roles contrast dramatically: for prey capture, the venom is required to paralyze quickly the prey; for defense, the venom should cause immediate pain and be toxic. We report here an investigation to determine the relationship between venom physiology and individual worker behavior relative to prey capture and to defense.

MATERIALS AND METHODS

Field investigation of *Termitopone commutata* (Roger) were conducted at the Embrapa Experimental Station, Moju, Pará, Brazil 48.768890 W 1.883890 S 16 m elevation on 27–31 December 2007. The area is undisturbed older secondary growth rain forest. A column of ants was followed to a colony of *Syntermes* sp. which was raided, after which the workers carrying paralyzed termites were followed back to their nest. A sample of 19 ants was obtained from the outward bound raiding column for venom analysis. The following day all individuals in the colony were sampled and colony individuals categorized as: *raiders*, workers taken from the raiding column; *defenders*, workers that vigorously rushed out of the nest when disturbed and attempted to sting the investigators; *nest workers*, workers that retreated when the colony was being excavated and made no attempt to defend the colony; *alate females*; and *males*. Larvae, pupae, and eggs were also recorded. Live ants were frozen and maintained at ca. -6°C for up to three days during which time they were dissected to obtain venom. Pure venom was obtained from the

frozen ants by the method of Schmidt (1995). In brief, frozen ants were thawed, their sting apparatuses removed to a spot of distilled water, the venom reservoir (minus filamentous glands) was pinched off and removed from the rest of the sting apparatus, rinsed with distilled water, and placed in clean distilled water. When about 50 individual reservoirs had been pooled in a single water drop, the venom was squeezed with pairs of forceps from each torn reservoir, the venom was dried over molecular sieve 5A (Supelco, Bellefonte, PA, USA) and stored in a freezer until used.

American cockroaches (*Periplaneta americana*) were used to determine the paralyzing ability of the venom. Swiss white mice were used for venom lethality analyses. Venom was dissolved in cockroach ringers modified from Weidler and Sieck (1977) and 1–4 μl was injected with a 5 μl microsyringe (SGE Analytical Science, Ringwood, Victoria, Australia) through the mesocoxal-sterneal membrane into groups of 6 cockroaches for worker ants and 4 cockroaches for alates. Paralysis, defined as the inability of the cockroach to move any legs, was recorded at 2 and 24 h. Cockroach death was defined as cessation of contractions of the dorsal artery (heart), complete loss of ability to move any mouthparts or the antennae, and the body turning brown. For lethality to mice, 0.15 M saline in volumes of 0.6% of the mouse body weight were injected *i.v.* into groups of 4 mice. LD_{50} values (24 hr) were calculated according to the method of Reed and Muensch (1938), with 95% confidence intervals (CI) determined by the method of Pizzi (1950), and means compared as in Woolf (1968; Chapter 19). The total paralytic and lethal activities of the venom from single ants are expressed as paralytic capacity and lethal capacity (Schmidt 1986), calculated by dividing the weight of venom per individual ant by the paralytic ED_{50} or the LD_{50} and is expressed in terms of weight of animal that would receive a median paralytic or lethal dose of venom from the sting of one average ant.



Fig. 1. Habitat of excavated *Termitopone commutata* colony showing extent of shallow nest tunnels and chambers delineated by grey sand (jar contains nest workers).

RESULTS

An outward bound raiding column approximately 5 m long and near the middle with 2–3 individuals abreast of *Termitopone commutata* was observed 16:15 local time. The column was followed to a colony of *Syntermes* sp. which was immediately raided. Within 10 minutes the raiding was essentially complete and the raiders returned to their colony carrying immobilized termites. In both the outward bound and return raiding party all individuals were moving in the same direction. The termite colony was located 82 m from the *Termitopone* colony.

The *T. commutata* nest had one main entrance with a tumulus 5 cm high and 15 cm in diameter, with several other entrances not surrounded by soil. Heavy daily rains likely washed away soil excavated from the colony, thus eliminating

most of the obvious signs of nest entrances. The nest encompassed an area approximately 1.5×1.75 m to a depth of 20 cm (Fig. 1). Numerous chambers, mostly at a depth of 15–20 cm were found throughout the nest. The total colony census, including the few workers that returned from the field over the next two days, is listed in Table 1. No eggs were found in the colony and relatively few larvae and pupae were

Table 1. Population of *Termitopone commutata* colony.

Individual category	n
Total workers	880
Alate ♀♀	85
♂♂	4
Queen(s)	1
Pupae	14
Larvae	43
Total adult population	970

Table 2. Worker caste specialization.

Worker caste specialization	n	% of population
(Raiders, estimate	100–150	11–17) ¹
Defenders + Raiders	308	35
Nest workers	572	65
Total worker population	880	100

¹ Visual estimate of raiding column size; raiders also considered defenders

present. Few males were in the nest. In contrast, alate females numbered almost a tenth as many as the workers. Only one obvious queen was present, though others might not have been recognized, as some alate females had shed their wings and egg laying had apparently ceased.

When the colony was disturbed, defending workers "boiled" from entrances and attacked investigators. These defenders were remarkably quick, agile, and readily stung. They maintained excellent grip on the skin and were hard to shake off. Stings were almost instant, rather painful and sharp, but not "burning" like honey bee or social wasp stings, produced little flare (redness surrounding the sting site) or wheal (white area immediately around sting entry site), and the pain lasted about 3–5 minutes ($n = 5$ stings between two investigators). An alate female that was picked up stung the first author and produced a reaction and pain about equal to that of a worker.

A large proportion of the colony workers actively attacked when the nest was disturbed. Of 880 workers, 308 actively attacked. The remainder of the population made no attempt to attack and quickly fled when uncovered. These nest workers

would attempt to sting in personal defense, but would not defend the colony as a whole. Defenders could not be distinguished from the previous day's raiders, who were presumed to become defenders when the nest was threatened. For this reason the count of raiders in Table 2 is an estimate based upon the raiding column length and density of individuals.

Venom activity.—The quantity of venom present among the female ant castes varied with both reproductive and behavioral caste. Raiders leaving the colony contained nearly twice as much venom as defenders (Table 3). Defenders, in turn, contained about 50 percent more venom than nest workers, and nest workers, in turn, contained almost twice the venom of alate females. These differences in venom quantity were obvious during the dissection process. The venom reservoirs of foragers were invariably round, full and turgid. Reservoirs of defenders varied from being half full to full (11 half full, 250 > half full). Venom reservoirs of many nest workers were collapsed and mostly empty, with a much smaller proportion being more than half full (199 mostly empty, 43 one quarter to half full, and 237 > half full). The degree of reservoir filling appeared connected to age of the worker. Many of the nest workers with mostly empty reservoirs were teneral and lighter in color, and most of the rest had clearly much softer integuments than defenders or raiders. The venom reservoirs of alate females appeared different from workers. Despite the alates being much larger than workers, their reservoirs appeared smaller in diameter

Table 3. Lethality and lethal capacity (LC) of *Termitopone commutata* venoms to mice.

Caste, or worker caste specialization	μg Venom per ant (n)	LD ₅₀ ($\mu\text{g}/\text{g}$) ¹	95% Conf. Interval	LC (g/sting) ¹	Rel. LC
Raiders	608 (14)	10.1	4.5–22	60.2	11.67
Defenders	366 (57)	11.3	5.4–24	32.4 **	6.28
Nest workers	237 (479)	11.3	5.7–23	21.0 ***	4.07
Alate ♀♀	131 (59)	25.4*	11–57	5.16 ****	1

¹ Probability of value different from raiders: * = <.025, ** = <.01, *** = <.001, **** = <<.001 (c-test of means, Woolf 1968).

Table 4. Paralyzing activity and paralytic capacity (PC) of *Termitopone commutata* venoms to cockroaches.

Caste, or worker caste specialization	ED ₅₀ , 2 h (µg/g) ¹	ED ₅₀ , 24 h (µg/g) ¹	LD ₅₀ , 24 h (µg/g) ¹	PC, 2 h (g/sting)	Prob different from: ¹		
					Raid	Defend	Nest W.
Raiders	61.7	113	160	9.85	-	-	-
Defenders	89.1	124	160	4.11	<.025	-	-
Nest workers	103	120	280	2.30	<.001	<.2	-
Alate ♀♀	113	113	160	1.16	<<.001	<.001	<.1

¹c-test of means, Woolf 1968.

than those of workers (9 mostly empty, 37 one quarter to half full, 13 full). Moreover, unlike the clear and transparent venoms of workers, alate venom appeared turbid and contained copious quantities of flocculent particles that did not readily dissolve in water.

The defensive value against large vertebrate potential predators of the venoms of *T. commutata* can be measured in terms of lethality (LD₅₀) and lethal capacity (LC) to mice. All three behavioral castes of workers exhibited essentially the same lethality, and all are significantly more lethal than alate female venom. Among the workers, significant differences in the potential "killing" power of the venom from a single ant, or lethal capacity, became apparent. The lethal capacities of the four venoms span an 11-fold activity range between raiders and alate females, with in between values for defenders and nest workers (Table 3).

The ability of *T. commutata* to capture prey by stinging and injecting venom is measured as the effective dose for paralysis of half of the stung population (ED₅₀) at 2 h or at 24 h. Although the ED₅₀ values at 2 h exhibited decreasing activities in a progression of raiders through defenders and nest workers to alate females, the differences were not significant. Some of the envenomed cockroaches recovered movement between 2 and 24 hours, as reflected in the higher amounts of venom required to maintain paralysis for 24 h compared to 2 h (Table 4). Even higher venom quantities were required to cause death in 24 h. As in defense, a stronger measure of the

paralyzing ability of an individual ant is the paralytic capacity (PC). Raiders have a significantly greater ability to paralyze prey than defenders, nest workers, or alate females. Defenders are not significantly better at paralyzing prey than nest workers, but are better than alate females (Table 4).

DISCUSSION

The colony of *Termitopone commutata* investigated in this report was the largest on report, containing nearly a thousand adults. Although *T. commutata* colonies likely will be less populous than its sister species *T. marginata*, colony biomass likely equals or exceeds that of its smaller relative. The colony life cycle and periods of brood rearing and alate production are not known for *T. commutata*. Based on the absence of eggs and the small number of larvae and pupae, the present colony appeared to have just completed a production cycle of worker and reproductive rearing. If other colonies follow this pattern is not known. Wheeler (1936) commented that males of this species were unknown and subsequent papers rarely mentioned males. The colony we excavated followed this pattern of producing few males relative to alate females (4 compared to 85), a feature that could be a result of individual colony variation, loss of males from recent mating flights, or simply that males are infrequently produced. Mill's observation in June of a colony containing approximately 75 males, 400 workers, and no alate females is consistent with large colony variation in the resources devoted to male

versus female reproductive production (Mill 1982). It also suggests that *T. commutata* might have brood rearing cycles and that our colony was at the end of a brood cycle, something also supported by the large proportion of teneral workers in the colony. The presence of large numbers of alate females in our colony suggests that the low male number was not the result of recent mating flights.

Raiding behavior and defensive behavior are readily distinguished from nest activities in workers of *Termitopone commutata*. Both tasks appear to be performed by specialist workers, not simply by random workers. Behavioral task specialization is a familiar topic in sociobiology and the genetic, hormonal and molecular bases of task specialization are becoming known (Robinson et al. 2008). Examination of the actual suitability of individuals to perform certain tasks has received little attention. For termite raiding and nest defense, the ability of *T. commutata* workers to perform the tasks optimally is dependent upon venom activity and quantity. Alate females, which neither defend the colony nor raid termites, possess venom significantly less lethal and less paralytic than workers. They also produce less venom than workers. This pattern is consistent with those of other species where reproductive and worker venoms have been compared (Schmidt and Schmidt 1985) and makes sense in terms both of resource allocation and behavioral needs.

Worker *T. commutata* behavioral castes contain different amounts of venom in their reservoirs, with raiders having the greatest amount, and nest workers the least. Unfortunately, the necessity to pool venom from many individuals to make the weight measurements precludes ability to test for significant differences among the groups. Both the lethality and paralyzing abilities of the venoms of the worker castes were similar, if not, identical. This finding suggests that venom composition and synthesis do not change with either age

or behavior of the individual, rather that venom production varies with age and, perhaps, with task specialization. The affect of venom production on ability of workers to perform raiding and defensive tasks is clearly seen in the measures of paralytic and lethal capacities. In both cases raiders or defenders have significantly greater ability to paralyze prey and to damage potential predators than nest workers. Thus, those workers that are physiologically best adapted for performing the tasks of prey capture and defense are those workers that actually perform the tasks, and those workers less able do not put themselves at risk performing tasks for which they are ill suited.

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